SUMMARY

of the doctoral thesis entitled:

THE IMPACT OF *BOLETUS EDULIS* ON HUMAN AND *SALMO SALAR* GUT MICROBIOTA, WITH THE TRANSFER OF ANTIBIOTIC RESISTANCE GENES IN *SALMO SALAR*

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Mushrooms have long been valued for their nutritional and medicinal benefits, with polysaccharides being a key component of their health-promoting properties. These complex carbohydrates found abundantly in mushrooms, serve as a food source for beneficial bacteria within the animal gut. By feeding these bacteria, mushroom polysaccharides help maintain a healthy microbiota, crucial for overall health, including enhanced digestion and improved immune function. Additionally, understanding how antimicrobial resistance (AMR) spreads is critical, as it poses a significant threat to human and animal health. This doctoral thesis explores the potential of *Boletus edulis* (BEE), a wild mushroom known for its culinary and medicinal properties, to influence gut microbiota in humans and Atlantic salmon (Salmo Salar). The study aims to evaluate BEE's impact on gut health, its prebiotic and antioxidant properties, and its role in modulating antimicrobial resistance (AMR) gene transfer using advanced artificial gut simulation systems. The thesis objectives were as follows: (i) conduct a systematic review by consulting the latest research in the domain to evaluate mushroom polysaccharides' impact on gut microbiota, immune responses, and antimicrobial resistance transfer; (ii) compare *Boletus edulis* extract with other mushroom species and utilize an advanced gastrointestinal simulator; (iii) test the effects of *Boletus edulis* extract on human gut microbiota affected by antibiotics using the artificial gut system; (iv) evaluate the prebiotic impact of different dosages of *Boletus edulis* extract on the gut microbiota of *Salmo Salar* using an artificial gut simulator; and (v) investigate the potential outcomes when a bacterium carrying a multi-resistant plasmid is introduced into a bacterial community and subjected to selective pressure through antibiotic treatment.

Based on these objectives The initial bibliographic chapters present a systematic review of recent studies in the relevant field, encompassing the influence of mushroom polysaccharides on human and Atlantic salmon gut microbiota. These chapters also emphasize the significance of using artificial gut systems to evaluate prebiotic and probiotic effects on a wide array of microbes that impact host health. The reliability of these in vitro systems in simulating antimicrobial resistance (AMR) spread is also highlighted.

The first chapter extensively reviews the role of mushroom polysaccharides, particularly their impact on gut microbiota composition and health in both humans and salmon. The utilization of artificial gut systems is explored in detail, demonstrating their effectiveness in identifying prebiotic and probiotic effects across diverse microbial populations. These systems provide valuable insights into the interactions between dietary components and gut microbiota, aiding in the development of targeted health interventions.

Following this, the second chapter investigates the genomic insights into bacterial gene transfer mechanisms. Traditional horizontal gene transfer processes such as conjugation, transformation, and transduction are discussed alongside newly discovered mechanisms like transposition and vesiculation. This chapter elucidates how bacteria disseminate genetic diversity, contributing to the spread of antimicrobial

resistance. By integrating these findings, the review underscores the critical role of genomic studies in understanding and mitigating AMR spread, thus informing future research and intervention strategies.

This first research chapter evaluates the antioxidant benefits of various mushroom extracts and their effects on human gut microbiota using an advanced gastrointestinal simulator. Recognizing the significant health benefits and bioactive compounds in mushrooms, this study focuses on extracting these compounds to maximize their health potential. The selected mushrooms include *Boletus edulis*, *Cordyceps militaris*, *Ganoderma lucidum*, *Inonotus obliquus*, *Hericium erinaceus*, and *Lentinula edodes*, chosen for their known antioxidant properties, polysaccharide content, and overall health benefits.

Optimal extraction conditions were established through a series of antioxidant tests, including DPPH radical scavenging, lipid peroxidation inhibition, and total polyphenol content determination. Experiments were conducted in triplicate for reliability. The study found that ethanol at 100°C significantly enhanced the lipid peroxidation inhibition of *Boletus edulis* extracts. Water was generally more effective for *Cordyceps militaris*, *Hericium erinaceus*, and *Ganoderma lucidum* in antioxidant activities, while ethanol was superior at higher temperatures for some species.

The research involved rigorous antioxidant testing to establish optimal extraction conditions for BEE, including DPPH radical scavenging activity, lipid peroxidation inhibition, and total polyphenol content determination. The findings revealed that ethanol extraction at 100°C significantly enhances BEE's lipid peroxidation inhibition. For other mushroom species like *Cordyceps militaris*, *Hericium erinaceus*, and *Ganoderma lucidum*, water proved more effective for antioxidant activities, while ethanol was superior at higher temperatures for some species.

Using sophisticated artificial gut simulators, the study assessed the distribution and abundance of bacterial populations in human and salmon intestinal models. These simulators accurately replicated the physical and biochemical conditions of the human and salmon gut, providing insights into the microbiota dynamics influenced by BEE.

The widespread use of antibiotics poses significant public health concerns, primarily due to the disruption of the human gut microbiota and the subsequent negative health consequences. This study aimed to evaluate the in vitro effects of a novel extract from *Boletus edulis* (BEE) on human gut microbiota, particularly in the context of antibiotic-induced dysbiosis. The investigation focused on several key parameters: cytotoxicity, the influence of BEE on cytokine levels (IL-6, IL-10, IL-1β, TNFα), and its capacity to modulate microbiota composition post-antibiotic treatment.

To achieve a comprehensive assessment, the study involved detailed microbiome analyses and evaluations of short-chain fatty acid (SCFA) synthesis. Concurrently, the study also determined the total polyphenol content, antioxidant capacity, and specific polyphenolic composition of the BEE extract. The results indicated that BEE effectively modulates the microbial landscape, promoting a healthier microbial balance and mitigating inflammatory responses.

Overall, the study highlights the promising role of *Boletus edulis* extract in restoring microbiota balance, reducing inflammation, and combating the adverse effects of antibiotic use. These findings support further exploration of BEE as a functional food ingredient with potential health benefits, particularly in mitigating antibiotic-induced dysbiosis and promoting overall gut health without the need for probiotic supplements.

The study investigates the effects of *Boletus edulis* extract (BEE), a novel prebiotic, on the microbial communities in the Atlantic salmon (Salmo Salar) gut using the SalmoSim in vitro simulation system. Given the rapid expansion of the salmon aquaculture sector, with significant exposure to abiotic and biotic stressors, there is a critical need for strategies to enhance feed conversion efficiency, reduce medical treatments, minimize waste, and improve fish quality. Prebiotics, particularly BEE, offer potential benefits by stimulating beneficial gut bacteria growth.

Three biological replicates of Atlantic salmon gut samples were processed under strict anaerobic conditions to maintain microbial integrity. Experimental diets containing 5% and 10% BEE were prepared, and SalmoSim bioreactors were inoculated with gut microbiota. The bioreactors were carefully controlled for temperature, pH, and nutrient flow to simulate the in vivo gut environment.

The study assessed microbial composition and fermentation production in response to BEE supplementation. Results showed significant microbial community shifts, particularly an increase in beneficial Bacillus species in the low-dose BEE group and dominance of Vibrio in the high-dose group. Additionally, there was a notable increase in acetate production, essential for gut health and metabolic functions, while control groups exhibited higher levels of butyrate and 3-methylbutyrate. BEE supplementation also effectively reduces ammonia levels, crucial for water quality and reduced toxicity in aquaculture systems.

The findings suggest that BEE can modulate the intestinal microbiota, enhance beneficial bacterial populations, improve short-chain fatty acid production, and lower ammonia levels, making it a valuable dietary supplement for promoting fish health and sustainability in aquaculture. Future studies should focus on optimizing BEE dosage and evaluating long-term in vivo impacts to fully harness its benefits for aquaculture applications.

The development, progression, and dissemination of antimicrobial resistance (AMR) is influenced by interlinked human, animal, and environmental ecosystems, posing severe risks to human health. Conjugative plasmid transfer drives the rapid dissemination of AMR among microbial populations. Mitigating antibiotic resistance spread requires an understanding of the dynamics of AMR transfer among microbial communities, as well as the role of various microbial taxa as potential reservoirs that promote long-term AMR persistence. In the last research chapter, we employed Hi-C, a high-throughput, culture-free technique, combined with qPCR, to monitor the carriage and transfer of a multidrug-resistant plasmid within an Atlantic salmon in vitro gut model during florfenicolultreatment, a benzene sulfonyl antibiotic widely deployed in fin-fish aquaculture. Microbial communities from the midgut (pyloric caeca) of three healthy adult farmed salmon were inoculated into three bioreactors developed for the SalmoSim gut system. The model system was inoculated with an Escherichia coli strain ATCC 25922 carrying plasmid pM07-1 and treated with florfenicol at a concentration of 150 mg/L fish feed media for five days before a washout/recovery phase. Hi-C and metagenomic sequencing identified numerous transfer events, including Gram-negative and Gram-positive taxa and, crucially, widespread transfer and persistence of the plasmid in the absence of florfenicol. Our findings highlight the role of commensal teleost gut floRa as a reservoir for AMR, and our system provides a model to study how different treatment regimes and interventions may be deployed to mitigate AMR persistence.The general conclusions chapter, derived from the other experimental chapters, is supplemented with a series of recommendations, both at the level of fundamental research and in terms of proposals for continuing certain applied aspects.

In conclusion, this thesis underscores the significant impact of *Boletus edulis* on gut microbiota and its potential applications in human health and sustainable aquaculture. The findings advocate for further in vivo studies, dose optimization, and exploration of BEE's mechanistic effects on gut health and immune modulation. Additionally, the study emphasizes the need for strategic interventions to mitigate AMR spread in aquaculture, leveraging the natural properties of functional foods like *Boletus edulis*.